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Clones, hermaphrodites and pregnancies: nature's oddities offer evolutionary lessons on reproduction

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Abstract

I love the term 'natural history' because it encapsulates the sentiment that nature's operations have evolutionary etiologies. Charles Darwin was a natural historian *par excellence* and his elucidation of natural selection, artificial selection, and sexual selection fundamentally changed how scientists interpret the origins of biological features previously ascribed to sentient craftsmanship by supernatural agents. Darwin's insights on evolutionary forces grew from his exceptional knowledge of natural history, yet two key topics steeped in natural history – sex and reproductive genetics – remained poorly understood (and probably even shunned) in Darwin's Victorian era. That situation changed dramatically in the latter half of the 20th century with societal awakenings about sexuality that also happened to coincide with the introduction of molecular parentage analyses that unveiled a plethora of formerly hidden 'sexcapades' throughout the biological world. Here I summarize some of the evolutionary revelations that have emerged from selection theory as applied to genetic and phylogenetic information on clonality, hermaphroditism, and pregnancy, three procreative phenomena that are relatively rare in vertebrate animals and thus offer alternative evolutionary perspectives on standard reproductive modes. Collectively, these three peculiarities of nature illustrate how the abnormal in biology can enlighten evolutionary thought about the norm.

Introduction

In the inaugural Thomas Henry Huxley (THH) Review for the *Journal of Zoology*, Birkhead (2010) provided a historical and contemporary account of post-copulatory sexual selection – the mere existence of which evolutionary biologists had failed to appreciate until late in the 20th century. In the second THH Review, Davies (2011) addressed another reproductive topic: brood parasitism. I am honored to author the third THH Review, in which I intend to follow Birkhead's and Davies's eloquent leads by addressing three additional areas of reproductive biology that until recently had received relatively scant attention in the evolutionary literature on vertebrate procreation. These are clonal reproduction (asexuality), hermaphroditism (reproduction by dual-sex individuals), and viviparity (pregnancy or live-bearing), all of which depart from their more prevalent opposites: sexual reproduction, gonochorism (separate-sex procreation) and oviparity (egg-laying), respectively. These topics are huge, so my plan is to extract some key evolutionary insights that have emerged from genetic appraisals of backboned animals (as well as various invertebrates and plants) that display these reproductive syndromes. The unifying theme of this overview is that exceptional phenomena in biology can beam novel light onto genetic conditions that are far more standard. THH was Darwin's staunch defender and spokesperson. I have no such advocate, so this review is also an unabashed attempt to advertise my recent trilogy of books (Avise, 2008, 2010, 2012) on peculiar reproductive modes. Readers may wish to consult those three works for much more evolutionary information about clonality, hermaphroditism and pregnancy than can be presented in this current synopsis.

As recounted by Birkhead (2010), sex and animal mating systems remained mysterious and were little discussed by Darwin, perhaps because of pervasive Victorian prudery (Birkhead, 1997), but also because very little was known at that time about the cellular or genetic mechanics of sexual procreation (Smith, 1998). Indeed, the realization that one spermatozoan cell and one ovum normally must unite to initiate embryonic development was one aspect of an emerging cell theory that had just begun to crystallize in the mid-1800s as a key adjunct to Mendel's (1865) revolutionary discoveries about hereditary transmission. Darwin could not have presaged that the emergence of anisogamy (the disparity in size and mobility between male and female gametes) early in the history of multicellular life would later become appreciated as one of the 'major transitions in evolution' (Maynard Smith &

Szathmáry, 1995). Indeed, anisogamy is now seen not only as the universal basis for defining maleness and femaleness in nearly every sexual species, but also as being the ultimate root of many evolutionary 'battles between the sexes' over optimal reproductive tactics by males versus females. Given the social climate of the mid-1800s, coupled with the paucity of information about the genetic bases of sex and sexuality, it is little wonder that Darwin declined to speculate unduly about the diverse sexual modes and alternative mating lifestyles of animals.

In Darwin's era and throughout the following century (well into the 1970s), essentially all inferences about animal reproductive activities in nature came from behavioral observations often coupled to evolutionary interpretations based on particular ecological or mating-system theories (e.g. Fisher, 1930; Bateman, 1948; Ford, 1964; Williams, 1966; Lack, 1968; Emlen & Oring, 1977; Krebs & Davies, 1978). Beginning in the late-1960s, however, a succession of increasingly powerful molecular techniques were introduced that soon permitted direct genetic appraisals of biological parentage (and hence of genetic mating systems) in natural populations (Avise, 1994), and also facilitated evolutionary reconstructions of the phylogenetic histories of alternative reproductive practices across species and higher taxa (Harvey *et al*., 1996; Avise, 2006). These genetic and phylogenetic analyses opened everyone's eyes to a plethora of reproductive shenanigans (including post-copulatory sperm competition) that had remained largely hidden or otherwise outside the spatial or temporal purview of even the most attentive field naturalists of earlier eras. These new sources of empirical information also rejuvenated interest in evolutionary theories about animal mating systems and reproductive behaviors (e.g. Trivers, 1972; Smith, 1984; Arnold & Duvall, 1994; Birkhead & Møller, 1998; Lucas & Simmons, 2006), which in turn gave further impetus to empirical studies in a synergism that continues to energize modern research in natural history and comparative reproductive biology.

In addition to being 'Darwin's bulldog', THH was an accomplished comparative biologist in his own right, so I would like to think that he (and Darwin) would be sympathetic to the integrative approach I am attempting here. The following sections introduce revelations that have emerged from comparative evolutionary vantages on three classes of nature's reproductive oddities: clones, hermaphrodites and pregnancies.

Vertebrate clonality

Background

Approximately 100 extant species of vertebrate animals (0.1% of the total) consistently reproduce without the benefit of sex (Dawley & Bogart, 1989). Darwin himself was aware of the phenomenon of 'virgin birth', as evidenced by a passage from his 1868 book (Darwin, 1868; p. 352): 'the now well-ascertained cases of parthenogenesis prove that the distinction between sexual and asexual generation is not nearly so great as was formerly thought, for ova occasionally, and even in some cases frequently, become developed into perfect beings, without the concourse of the male'. We now know that a diverse miscellany of reptilian, amphibian and piscine evolutionary lineages consist solely of females who reproduce by parthenogenesis or related reproductive modes that entail little or no genetic participation by males and sperm. These all-female lineages sometimes are referred to as clonal 'biotypes' (because the standard definitions of sexual biological species hardly apply). They perpetuate themselves by producing unfertilized ova that develop directly into daughter individuals who will carry on these traditions of sexual abstinence.

Genetic approaches

To address the evolutionary origins and genealogical histories of such vertebrate clones, geneticists use cytonuclear analyses that appraise cytoplasmically housed mitochondrial (mt) DNA sequences in conjunction with genotypic data (such as those traditionally revealed in allozyme surveys) from multiple unlinked nuclear loci. In the last 20 years, 'cytonuclear genetic signatures' (Avise, 2001) have been used to unveil both the modes of origin and the subsequent evolutionary histories of nearly all known unisexual vertebrate lineages.

Mt analyses (even alone) are of special relevance for such clonal taxa (Avise, Quattro & Vrijenhoek, 1992) because the genealogical history of mt transmission is, in principle, one and the same as a biotype's entire organismal phylogeny, which consists of nothing other than matrilineal ancestry. This contrasts dramatically with the standard situation in sexual taxa where the matrilineal genealogy is only a miniscule fraction of a species' total hereditary legacy, most of which is ensconced instead in the nuclear genome whose alleles have been transmitted across the generations via both males and females through multitudinous unlinked nuclear 'gene trees' (Avise, 2000) that inevitably differ topologically from locus to locus because of the Mendelian rules of segregation and independent assortment. Although the 'blessedly celibate' (Dawkins, 1995) mtDNA molecule can simplify the genealogical bookkeeping in any animal species (sexual or otherwise), these ledgers of matrilineal ancestry are especially informative for celibate unisexual taxa whose entire genetic pedigrees are also basically clonal.

Six broader evolutionary revelations

(1) New 'species' can arise via hybridization

Conventional evolutionary wisdom is that new vertebrate species normally arise either via a splitting of lineages (cladogenesis) or by gradual transformations through time in ancestral-descendant series of populations (anagenesis). However, all known vertebrate taxa that are constitutively clonal clearly arose via interspecific hybridization events between progenitor species with standard sexuality. The basic suspicion is that normal meiotic and sexual operations became disrupted in hybrid offspring in ways that precipitated each evolutionary transition to ameiotic asexual reproduction. For

several clonal vertebrate taxa, researchers have used molecular markers to help clarify some of the detailed cytogenetic mechanics of unisexual origins (Uzzell, 1970; Dawley & Bogart, 1989; Quattro, Avise & Vrijenhoek, 1992*a*).

Molecular markers have also been used to pinpoint the sexual species and the direction(s) of the original cross(es) that produced each unisexual biotype (e.g. Avise *et al*., 1991). To pick just a few examples, the diploid parthenogenetic rock lizard *Darevskia rostombekovi* of central Europe apparently arose via a single cross between a sexual *D. raddei* female and a sexual *D. portschinskii* male (Moritz, Wright & Brown, 1992; MacCulloch *et al*., 1997), whereas some other unisexual taxa such as parthenogenetic lizards *Menetia greyii* (Adams *et al*., 2003) and hybridogenetic fishes named *Poeciliopsis monachalucida* (Quattro, Avise & Vrijenhoek, 1991) each encompass multiple evolutionary lineages that originated via separate hybridization events. In the *Poeciliopsis* case, the hybridizations that give rise to unisexual biotypes appear to be ongoing. For these unisexual fish, the interpretation is that each such event genetically 'freezes' a new clonal genotype (Vrijenhoek, 1984), which if lucky might happen to fill an open ecological niche. Thus, overall, many biotypes are generated but probably only a few persist for very long.

Another revelation about unisexual origins is that the sexual progenitors that hybridized to produce each clonal lineage usually are not sister species but instead belong to different branches of the phylogenetic tree for that taxonomic genus. Two hypotheses (not mutually excusive) have been advanced for this observation. Under the balance hypothesis, parthenogenesis can arise only when the genomes of parental species are divergent enough to disrupt meiosis in hybrids yet not so divergent as to seriously compromise hybrid viability or fertility. By contrast, the phylogenetic constraint hypothesis posits that genetic peculiarities predispose particular parental species to produce unisexual lineages following hybridization.

To the extent that unisexual vertebrate biotypes can be deemed valid species (as they are in the formal taxonomies as well as in the sense that they are reproductively isolated from one another), they challenge the standard evolutionary paradigm that homoploid speciation invariably is a process of lineage diversification rather than lineage amalgamation or anastomosis via hybridization (Mallet, 2007).

(2) New species can arise suddenly

Both sympatric and allopatric scenarios of animal speciation typically envision slow and gradual genetic transformations of populations, even when vicariant events in the physical environment are sudden. But unisexual vertebrate taxa break this evolutionary rule because each biotype emerges quickly (in one or a few generations) from the two (or sometimes more) sexual species that had hybridized to produce it (Dawley & Bogart, 1989; Vrijenhoek, 1994). Thus, in a temporal sense, the emergence of many parthenogenetic animal species parallels the rapid emergence of many allopolyploid plant species that also have arisen following interspecific hybridization events.

(3) Some clones can achieve evolutionary longevity

Conventional wisdom holds that genetic recombination (typically via sexual reproduction in multicellular organisms) is necessary for continued adaptability to changing environments and for the long-term evolutionary persistence of any species. To assess the evolutionary ages of vertebrate clones, researchers have generated and provisionally dated phylogenetic trees (typically from mtDNA sequences and molecular-clock calibrations) for many unisexual taxa and their sexual relatives. Results proved generally consistent with the standard thesis that asexual lineages have short evolutionary durations, but there do seem to be some exceptions. For example, Quattro, Avise & Vrijenhoek (1992*b*) used a large geographic range and high post-formational cytonuclear genetic diversity to estimate that a monophyletic biotype of the unisexual fish *Poeciliopsis monacha-occidentalis* is about 60 000 years old. Although Maynard Smith (1992) rightly noted in a commentary that 60 000 years 'is but an evening gone' in evolutionary time, it does seem clear that at least some vertebrate clones are far more persistent than formerly realized. In any event, this and other longevity estimates for various unisexual vertebrate lineages all pale in comparison with the ancient origins suspected for some invertebrate parthenogenetic lineages that seem to have survived without sex for tens of millions of years (Mark Welch, Mark Welch & Meselson, 2004; Domes *et al*., 2007; Heethoff *et al*., 2007).

(4) Some unisexual lineages are quasisexual or hemiclonal

Female parthenogens truly are sexually chaste, but females in gynogenetic and hybridogenetic vertebrate taxa might be deemed only 'semichaste'. As under parthenogenesis, a gynogenetic female reproduces clonally except that sperm from males of a related sexual species are required to initiate cellular divisions in her unreduced ova. A sperm cell does not actually fertilize an egg but merely stimulates it to begin dividing. Thus, a gynogenetic female in effect 'sexual parasitizes' a foreign male who receives no genetic payoff for his sexual services. Hybridogenesis is another peculiar mode of reproduction with elements of both clonality and sexuality. Oddly, a hybridogenetic female produces reduced (haploid) ova that carry only the chromosome set that she had received from her mother. Each ovum is fertilized by a sperm cell thereby reestablishing the diploid condition in the resulting offspring. However, the exclusion of the paternally derived set of chromosomes during oogenesis in the daughter means that a sexually parasitized male can be a genetic father but he cannot be a grandfather or otherwise pass copies of his genes to future generations. Thus, the intact (non-recombined) set of maternal chromosomes is the primary clonal component of the 'hemiclonal' system of unisexual taxa that display hybridogenesis. Finally, even more genetic complications arise in other 'kleptogenetic' all-female lineages that occasionally incorporate or 'steal' foreign nuclear DNA from related sexual species (Bogart *et al*., 2007).

The obligate involvement of heterospecific males in gynogenetic and hybridogenetic reproduction places ecological, behavioral, distributional and coevolutionary constraints on sperm-dependent unisexual lineages beyond those endured by sperm-independent parthenogens.

(5) Clonality can be intra- as well as intergenerational

Many vertebrate as well as invertebrate animals occasionally produce monozygotic twins, triplets, etc. The production of clonemate (genetically identical) siblings is known as polyembryony, which thus is an intra- rather than intergenerational expression of clonality. Even sporadic polyembryony might seem at face value to be an unwise reproductive tactic that has been likened to a reproductive raffle in which parents purchase multiple lottery tickets (different progeny) with the identical number or same multilocus genotype (Williams, 1975). Even more surprising is the fact that a few sexual species produce clonemate broods consistently and exclusively. The multiple offspring in any polyembryonic litter have arisen from sexual reproduction (meiosis followed by syngamy), so the recombined genotype that they all share is distinct from those of both parents and has never before been 'field-tested' for proper performance. Nevertheless, constitutive polyembryony is a standard mode of reproduction in diverse invertebrate taxa (Craig *et al*., 1997) and also in the one vertebrate clade: armadillos in the genus *Dasypus* (Prodöhl *et al*., 1996; Billingham & Neaves, 2005). A long-standing evolutionary enigma has been why armadillos (or indeed why any animal species) would routinely fabricate clonemate sibships each with a photocopied but unproven genotype.

Many polyembryonic invertebrates are endoparasites that spend part of their life cycle within a host's body (Strand, 1989). Wasps that parasitize moths provide illuminating examples. A moth egg is the typical site into which the female wasp deposits a fertilized egg that later divides polyembryonically within the developing host caterpillar (Grbic, Nagy & Strand, 1998). Polyembryony may make evolutionary sense in this circumstance because the parasite faces a temporary space bottleneck (the host egg) that later will expand into a spacious environment (the caterpillar body) upon which multiple parasitic larvae can feast.

An analogous scenario may apply to the evolution of constitutive polyembryony in *Dasypus* armadillos (Loughry *et al*., 1998). In these species, the initial reproductive bottleneck is an oddly configured uterus with only one blastocyst implantation site. Polyembryonic divisions early in a female's pregnancy then give rise to multiple clonemate offspring that will be housed within her later-enlarged uterus. Thus, for parasitic wasps and armadillos alike, polyembryony might be interpreted as an opportunistic reproductive tactic that makes the best of the available situation for both parental and offspring genetic fitness. In each case, a severe constraint on offspring numbers exists at the outset of each 'pregnancy', but a spacious developmental niche (host caterpillar and female uterus, respectively) arises later that can be exploited by multiple polyembryos. Furthermore, for the co-housed siblings, competition should be minimized and cooperation fostered because the broodmates are also clonemates (Hamilton, 1964; Hardy, 1995; Giron *et al*., 2004). If these speculations about the adaptive significance of polyembryony are correct, they might conform to the broader notion that polyembryony tends to evolve when offspring have more information about optimal clutch size than do their parents (Godfray, 1994; Craig *et al*., 1997). When progeny are in the best position to assess the environmental resources available to them, polyembryony would be selectively advantageous to them (as well as to the genetic fitness of their parents) if the polyembryos can adjust the extent of their clonal proliferation accordingly. In any event, constitutive polyembryony again illustrates how biological oddities can instruct broader evolutionary thought.

(6) Inbreeding can also produce clones

This last point about clonality provides an obvious segue into the next section that will expand on the topic of hermaphroditism. Inbreeding (the mating of kin) tends to decrease genetic variation in a sexual pedigree and in the extreme becomes another potential evolutionary route to 'clonality'. Selfing is a most intense form of inbreeding. Consider, for example, the mangrove killifish (*Kryptolebias marmoratus*), nature's only hermaphroditic vertebrate that routinely mates with itself (self-fertilizes). Each mature dual-sex individual houses an internal ovotestis that simultaneously produces ova and sperm that unite within the fish's body before the zygotes are shed to inaugurate the next generation of self-fertilizers. When continued generation after generation, selfing soon leads to the emergence of genetic strains each composed of multiple individuals so genetically uniform as to be, in effect, clonally identical (Harrington & Kallman, 1968; Turner *et al*., 1992; Mackiewicz *et al*., 2006*a*).

For these fish and for some invertebrate and plant species that also self-fertilize routinely, researchers have proposed at least two fitness advantages that could ameliorate or even outweigh the oft-severe problem of inbreeding depression (Charlesworth & Charlesworth, 1987; Frankham, Ballou & Briscoe, 2002). One potential benefit is the opportunity to propagate clonal copies of genotypes co-adapted to local habitat conditions (Allard, 1975). A second benefit is fertilization insurance attributable to the fact that selfers are procreatively self-sufficient because they need not find a mate in order to reproduce (Baker, 1955). This latter advantage is the leading explanation for the adaptive significance of selfing in mangrove killifish, and it is also consistent with an observed association in plants and invertebrate animals between weediness (colonization potential) and the capacity for self-fertilization (Longhurst, 1955; Baker & Stebbins, 1965).

Vertebrate hermaphroditism

Background

Approximately 99% of extant vertebrate species consist of individuals that function either as male or female, but not

both. These are gonochoristic (separate-sex) species. Most of the remaining species include at least some hermaphroditic individuals with dual sexual functions. In species that are sequentially hermaphroditic, an individual might begin life as a male and later switch to a female (protandry), or it might be female first before transforming to a male (protogyny), or it might switch back and forth repeatedly between male and female. In vertebrate species with simultaneous hermaphroditism, by contrast, an individual may function both as male and female at the same time, in which case a dual-sex adult typically reproduces by outcrossing with other individuals. As mentioned above, however, *K. marmoratus* is a striking exception because each hermaphrodite typically self-fertilizes.

All of these hermaphroditic phenomena in fishes find near-perfect analogues in plants and invertebrate animals that also express various forms of dual sexuality. For example, approximately 95% of all species of flowering plants (angiosperms) include at least some dual-sex individuals as do more than 50 000 invertebrate animal species. Darwin was well aware of cosexual creatures, having conducted research and written books on hermaphroditic species of plants (Darwin, 1876, 1877) and marine invertebrates (Darwin, 1851, 1854). In general, however, the reproductive lifestyles of dual-sex organisms can seem quite foreign to us humans, who are more accustomed to thinking of the two sexes being housed in separate bodies.

Genetic approaches

Nuclear Mendelian markers such as allozymes or microsatellite loci are suited well for estimating otherwise cryptic mating-system parameters including selfing versus outrossing rates in hermaphroditic taxa. A substantial cottage industry in biology is devoted to characterizing alternative genetic mating systems (Clegg, 1980; Vogler & Kalisz, 2001) and interpreting their adaptive significance (Charnov, Maynard Smith & Bull, 1976; Charlesworth & Charlesworth, 1979) in taxa with dual-sex individuals. On a related empirical front, phylogenies reconstructed from DNA sequences are now used routinely as genealogical backdrop for deciphering evolutionary transitions between alternative sexual systems such as dioecy versus cosexuality in plants (e.g. Charlesworth, 2002; Sakai *et al.*, 2006), gonochorism versus hermaphroditism in animals (Mank, Promislow & Avise, 2006; Avise & Mank, 2009), and different forms of hermaphroditism such as protogyny versus protandry (Allsop & West, 2003). Such analyses are all part of a broader evolutionary enterprise sometimes referred to as 'phylogenetic character mapping' or PCM (Avise, 2006).

On the conceptual front, a major advance was the elaboration of a 'sex allocation' theory (Charnov, 1982) that uses fitness arguments to identify the optimal allocation of finite resources to male versus female functions in dual-sex individuals, given various ecological constraints and life-history trade-offs. Sex allocation theory has guided much of the evolutionary research on dual sexuality (West, Herre & Sheldon, 2000) and indeed has been hailed as 'a touchstone in the study of adaptation' (Frank, 2002).

Six broader evolutionary revelations

(1) Some species display mixtures of cosexuality and separate sexes

Rather than being mutually exclusive, gonochorism (i.e. dioecy) and hermaphroditism are merely signposts along a continuum of sexual systems. For example, many plant species and a few invertebrate animals consist of mixtures of dual-sex and unisex individuals, with the unisex specimens being males and females, respectively, in species that by definition are androdioecious or gynodioecious. Furthermore, the frequencies of both cosexual and unisexual individuals in dual-sex species can vary from rare to common. A few plant populations are even trioecious, consisting of mixtures of pure male, pure female and hermaphroditic individuals.

(2) Phylogenetic transitions to and from hermaphroditism differ across taxa

For invertebrate animals, hermaphroditism probably is a derived condition both overall and in many lower-level taxa (Eppley & Jesson, 2008), whereas the reverse trend prevails in plants where hermaphroditism often is the ancestral state from which dioecy has evolved on many separate occasions (Donoghue, 1989). Thus, even as invertebrate biologists strive to identify selective forces that might promote the evolution of hermaphroditism, botanists have wrestled with the opposite dilemma first posed by Darwin (1877): 'There is much difficulty in understanding why hermaphroditic plants should ever have been rendered dioecious'. Darwin suggested that 'if a species were subjected to unfavorable conditions . . . the production of the male and the female elements . . . might prove to be too great a strain on its powers, and the separation of the sexes would then be highly beneficial'. Aside from such ontogenetic challenges, botanists today also focus on dioecy's potential selective advantages (Vamosi, Otto & Barrett, 2003), which include inbreeding avoidance because dioecy enforces outcrossing (Charlesworth & Charlesworth, 1987; Husband & Schemske, 1996). However, for most botanists and zoologists alike, evolutionary transitions to and from hermaphroditism now are mostly viewed as part of a broader evolutionary challenge of understanding how each individual's genetic fitness might be affected by each reallocation of resources between male and female sexual functions (Goldman & Willson, 1986; Dorken & Mitchard, 2008).

Another modern topic involves deciphering transitional evolutionary conditions. For plants, PCM and other evidence indicate that evolutionary transitions to dioecy from cosexuality often occur along an evolutionary pathway that entails gynodioecy as an intermediate stage. For invertebrate animals, however, intermediate evolutionary states generally have been harder to identify, in part because androdioecy and gynodioecy are rare and probably transient conditions in animals.

(3) Sexual selection continues to operate in hermaphroditic species

Perhaps contrary to naive expectations, sexual selection (selective pressures arising from competition for mates or for opposite-sex gametes) does not cease with the evolutionary dissolution of the separate-sex condition. Instead, evidence of many sorts strongly implicates continuing pervasive roles for sexual selection in the evolution of sex-related phenotypes in hermaphroditic animals (Leonard, 2006) and dual-sex plants (Willson, 1990).

(4) Selfing versus outcrossing is an important fitness consideration

Dual sexuality opens a window of opportunity for self-fertilization that simply is closed to gonochoristic or dioecious species. But this option may or may not be exercised depending on the species and circumstance. For example, many hermaphroditic plant species have evolved mechanisms such as dichogamy (a temporal separation in an individual's production of male and female gametes), herkogamy (a physical separation of male and female gametes on a plant), and genetic self-incompatibilities, all of which can inhibit selfing, promote outcrossing, and thereby circumvent inbreeding depression. These mechanisms often are less than fully effective, however, with the net result that many dual-sex plant species display 'mixed-mating' systems with intermediate rates of selfing and outcrossing, and the same holds true for many invertebrate animals (Jarne & Auld, 2006). Species that show gynodioecy or androdioecy (or other categories of dual sexuality) also can have mixed-mating systems. The outcrossing component is guaranteed (assuming that pure males and pure females are reproductively successful), so the behavior of hermaphroditic specimens determines whether selfing (and hence mixed-mating) applies as well.

(5) Mixed-mating systems show convergent evolution

At least one vertebrate species – the mangrove killifish (*K. marmoratus*) – also shows a mixed-mating system of selfing and outcrossing (Mackiewicz *et al*., 2006*b*). Some populations of this species include functional adult males as well as the hermaphrodites with whom the males apparently outcross occasionally (Mackiewicz *et al*., 2006*a*,*b*,*c*). Thus, mixed-mating systems have evolved convergently not only in numerous plants and invertebrate animals but also in this one small vertebrate clade (Tatarenkov *et al*., 2009). In the case of *K. marmoratus* (as in many plants and invertebrate animals), the selfing component of the mixed-mating system undoubtedly offers hermaphrodites the significant evolutionary advantage of fertilization insurance. Indeed, given that multiple 'clonal' strains of extant mangrove killifish clearly have escaped the perils of intense inbreeding, at least over the short term, this androdioecious species with a mixed-mating system presumably enjoys some of the best of two worlds: outcrossing's long-term as well as short-term advantages (continued genetic health and adaptability through recombination), *and* selfing's immediate benefits (fertilization assurance and perhaps the intact propagation of locally adapted genotypes).

(6) Sequential hermaphrodites know when to switch sexual functions

Especially for animals that are sequential hermaphrodites, the most powerful evolutionary explanations for the ontogeny of sex change have come from a branch of sex-allocation theory known as the size-advantage hypothesis or SAH (Ghiselin, 1969; Warner, 1975, 1988), which basically predicts that sex change is favored by natural selection when an individual reproduces most effectively as one sex when small (and young) but as the other sex when larger (and older). Depending on the biology and ecology of a particular species, males might have a reproductive advantage when small and females when large, in which case protandry would be selectively favored; but in other species, females might reproduce better when small and males when large, in which case protogyny might tend to evolve. The empirical challenge has been to understand what biological conditions generally tip the scales in favor of individuals reproducing as dams versus sires at various size cohorts or age classes. For sequentially hermaphroditic fishes and invertebrates alike, SAH has made predictions about patterns of sex change that seem to be consistent with many observational and experimental tests.

Comparative pregnancy and brooding

Because humans are mammals with sexual reproduction, people are familiar with the concept of pregnancy, that is with the otherwise outlandish notion that one individual carries a genetically different individual inside its body for an extended period of time before expelling the latter through an orifice. If you are a man, you might feel relieved that this weighty reproductive imposition has been delegated to females in *Homo sapiens*; and if you are a woman, the thought of becoming pregnant might elicit any of a gamut of emotions ranging from joy to fear or loathing, depending on the circumstances.

One day when I was about 8 years old, I had an insight: God had arranged things equitably for men and women. A man could anticipate being drafted into 2 years of military combat whereas a woman might spend on average about 2 years of life in a state of pregnancy (which I imagined to be an equally unpleasant sentence). This childhood revelation is silly, but in some ways it was prescient. For my generation, about 60 000 young American men died and 160 000 were wounded in Vietnam; whereas across those same years (1959–1975) nearly 10 000 young women lost their lives in the United States and tens of thousands suffered enduring medical disabilities from complications of pregnancy (Kaunitz *et al*., 1985). Furthermore, on a global scale in recent decades, armed combat has claimed the lives more than half a million young men annually (GBAV, 2008); but 'maternal mortality' (defined as a mother's death related to pregnancy) likewise has exceeded 500 000 women per year (Hill *et al*., 2007). These

morbid statistics suggest that my childhood musings about the tribulations of the sexes contained a kernel of truth: young men and women have heavy but different crosses to bear. The statistics also remind us that that pregnancy is a focal time of death as well as birth.

Although nearly all mammals gestate embryos inside the dam's body, female pregnancy is far from universal in the biological world and there are even some species in which males alone become pregnant. Alternative gestational modes permit comparative analyses of how different expressions of pregnancy might impact the evolutionary ground rules for selection pressures on males versus females. With respect to sexual selection, pregnancy entails an asymmetric energetic investment in offspring by the two parents and thereby should have major consequences for the evolution of reproductive behaviors and mating systems. With respect to natural selection, pregnancy occupies a key intersection between the two major components of personal genetic fitness: survival and reproduction. Especially when a placenta physically connects parent with child, pregnancy also provides a uniquely intimate nexus between successive generations. Both of these biological junctures (between parent and child and between survival and reproduction) generate evolutionary conflicts of interest between a mother and her offspring that can be just as consequential for procreation as are conflicts between males over scarce resources and mates.

Background

Webster's dictionary defines pregnancy as 'having a child or other offspring developing in the body' whereas my *Chambers* dictionary describes the condition simply as being 'with child or young'. Both definitions can be relevant depending on the context. I will apply *Webster's* definition to animals such as mammals and some fish species in which a pregnant individual (usually a female but sometimes a male) carries embryos inside its body before giving birth to live young. This is viviparous 'internal pregnancy', regardless of the extent to which a parent offers resources other than brood space to its young. I will also take advantage of the ambiguity in *Chambers'* definition by extending the meaning of pregnancy to encompass situations in which a parent carries offspring on its body in what in effect becomes an 'external pregnancy'. I will even extend the notion of pregnancy to include oviparous nest-tending fishes in which the embryos that a parent supports are physically separate from the caretaker's body.

Genetic approaches

With respect to empirical studies of mating systems and sexual selection in the context of pregnancy, genetic parentage analyses based on highly polymorphic microsatellite markers have been popular. For any type of pregnancy, successful mating events (those that yield progeny) by the adult caregiver are relatively straightforward to deduce via molecular parentage analyses because embryos in each brood are physically associated with their pregnant sire or dam. For example, paternity in female-pregnant species can be determined by subtracting known maternal alleles from each offspring's diploid genotype, and thereby deducing which males had mated successfully with the dam of each assayed brood. By contrast, documenting mating behaviors by members of the non-pregnant sex is much more problematic because each such individual may have parented additional broods that were not included in the genetic assays (Jones & Ardren, 2003). Thus, the logistics of parentage analysis make molecular markers ideally suited for quantifying multiple paternity (polyandry by females) within the broods of female-pregnant species and multiple maternity (polygyny by males) within the broods of male-pregnant species, rather than the converse (Avise *et al.*, 2002; Avise & Liu, 2010, 2011).

With respect to the conceptual foundations of selection in the context of pregnancy, 'parental investment' theory (Trivers, 1972; Parker & Simmons, 1996) has been especially important as an adjunct to standard mating-system theories (e.g. Bateman, 1948; Orians, 1969; Emlen & Oring, 1977; Arnold & Duvall, 1994). One standard evolutionary train of thought is as follows: beginning early in the evolutionary history of multicellular sexual life, anisogamy promoted gametic retention by females and gametic dispersion by males, and these gender-specific proclivities in turn often promoted within-female syngamy (internal fertilization), which in turn predisposed the female sex to evolve pregnancy-like phenomena, which in turn makes females even more of a limiting reproductive resource compared with males, which further amplifies the evolutionary authority of females over reproductive affairs, which in turn further impacts the operation of sexual selection and thereby amplifies the proverbial 'battle between the sexes.'

Seven broader evolutionary revelations

(1) Pregnancy entails conflicts as well as cooperation

Pregnancy might seem to be the ultimate collaborative endeavor between individuals because (1) a mother and her fetus both have a vested personal interest in a successful outcome; and (2) so too does the father. Indeed, all three participants (sire, dam and fetus) would seem to share a mutual concern that progeny are born healthy after a productive incubation. On the other hand, each female mammal alone bears the physical burden of incubation and nursing whereas the sire may have little or no reproductive involvement beyond his original genetic contribution. Furthermore, in most sexual species, each family member has a unique genotype, implying that a gene's optimal tactic for self-perpetuation might depend to some degree on which individuals house that gene and any of its copies. Also, the selfish genetic interests of interacting organisms tend to be aligned only insofar as those individuals are related (Hamilton, 1964; Mock & Parker, 1997), and pairs of individuals in a nuclear family differ dramatically in their coefficients of genetic relatedness (r): a mother and her offspring normally share half their genes (r = 0.5) as do full sibs in a multi-birth litter; but

half-sib progeny share only one-quarter of their genes (r = 0.25), and a sire and dam typically are unrelated (r = 0.0).

For these and other reasons, each nuclear family is not simply a serene setting for harmonious interactions, but rather it can be an evolutionary minefield of oft-competing genetic fitness interests, both inter- and intragenerational (Trivers, 1972, 1974; Hausfater & Hrdy, 1984; Parmigiani and Vom Saal, 1994; Hudson & Trillmich, 2008). Furthermore, many of these conflicts play out forcefully within the mammalian womb. Thus, pregnancy becomes an evolutionary theatre for intergenerational conflict over parental resources – each offspring is under selection to seek as many maternal resources as possible (limited only by any negative effects on its inclusive fitness that such demands impose on copies of its genes carried by its kin), whereas a dam can be expected to resist excessive demands by the fetus. The net result of each such evolutionary 'tug-of-war' (Moore & Haig, 1991) between mother and child is some ontogenetic balance in which each offspring must settle for fewer maternal resources than it ideally might wish and a mother surrenders more resources than she otherwise might prefer. But by evolutionary reckoning, any such maternal–fetal compromise during or after a pregnancy is less the result of a harmonious mutualism than it is an outcome of conflict mediation (Haig, 1993, 1999, 2010; Nesse & Williams, 1994). Of course, maternal–offspring relations entail elements of cooperation as well as conflict; these two categories of interaction need not always be interpreted as mutually exclusive (Strassmann *et al*., 2011).

(2) Genomic imprinting is a remarkable ramification of pregnancy

Selective pressures that pregnancy promotes sometimes have led to outcomes that catch researchers totally off-guard. One such phenomenon is genetic imprinting: a situation in which a gene is expressed in progeny when inherited from one parent but not from the other (Solter, 1988). In such cases, a gene can have very different effects on offspring (and therefore on the course of a pregnancy) depending on whether it was transmitted via the dam (egg) or sire (sperm). Genetic imprinting in animals appears to be confined mostly to viviparous mammals, but the phenomenon also is common in plants (Feil & Berger, 2007). In recent years, scientists have discovered imprinted genes in many marsupial and placental mammals, including *Homo sapiens*, where imprinting has been documented at approximately 100 loci to date. Mechanistically, imprinting usually results from the addition of methyl ($-CH_3$) groups to particular nucleotides during the production of male or female gametes, resulting in the specific inactivation of either maternal or paternal genes in offspring (Reik & Walter, 2001). The terms padumnal and madumnal refer to paternally and maternally derived alleles in offspring, so genetic imprinting essentially involves altered expressions of madumnal or padumnal alleles (Haig, 1996).

Haig (1993) introduced evolutionary interpretations for genetic imprinting (and for various other expressions of conflict during mammalian pregnancy) when he wrote:

> The effects of natural selection on genes expressed in fetuses may be opposed by the effects of natural selection on genes expressed in mothers. In this sense, a genetic conflict can be said to exist between maternal and fetal genes. Fetal genes will be selected to increase the transfer of nutrients to their fetus, and maternal genes will be selected to limit transfers in excess of some maternal optimum. Thus a process of evolutionary escalation is predicted in which fetal actions are opposed by maternal countermeasures. The phenomenon of genomic imprinting means that a similar conflict exists within fetal cells between genes that are expressed when maternally derived, and genes that are expressed when paternally derived.
>
> (Haig, 1993)

Haig's seminal idea has become known as the conflict hypothesis or the kinship hypothesis for genetic imprinting and it still remains the leading evolutionary explanation for the imprinting phenomenon.

Unfortunately, these strategic battles between madumnal and padumnal genes *in utero* come not without serious medical consequences, especially for embryos that are caught in the evolutionary crossfires (e.g. Haig, 2004). For example, Frank & Crespi (2011) suggest that such intragenomic conflict may affect the regulation of embryonic growth in ways that can precipitate various pathologies such as some cancers as well as psychiatric disorders including some cases of autism and schizophrenia. These authors view evolutionary-genetic conflict as sexual antagonism that can lead to pathologies whenever opposing genetic interests that normally are precariously balanced become unbalanced for any reason. Burt & Trivers (2006) have extended this kind of evolutionary argumentation about intergenic strife to a broad spectrum of otherwise puzzling empirical properties of sexual genomes.

(3) Not all aspects of pregnancy have been shaped by natural selection

Even among mammals, various expressions of pregnancy sometime have and sometimes have not been forged by natural selection. For example, embryonic diapause wherein a delay occurs between fertilization and implantation is a polyphyletic condition that clearly demands an adaptive explanation (related in this case to differences in optimal times for mating vs. birthing); whereas sporadic polyembryony (the occasional production of monozygotic twins) is an idiosyncratic happening that almost certainly is not adaptive *per se*. And other expressions of pregnancy (such as constitutive dizygotic twinning in marmosets and tamarins; Signer, Anzenberger & Jeffreys, 2000) have some biological elements that do and other elements that probably do not require adaptive explication.

(4) Pregnancy is not always a black-or-white condition

Viviparity ('live-bearing') is often viewed as the antithesis of oviparity (egg-laying), but in fact these two reproductive

modes are just signposts along a continuum of gestational systems. Many fish and other vertebrate species are ovoviviparous, meaning that gravid females carry internally fertilized eggs that hatch within a dam before she gives birth to live young. Furthermore, a remarkable diversity of gestational phenomena in the biological world gives added testimony to the sentiment that pregnancy is not invariably the all-or-nothing syndrome that we mammals otherwise might assume. For example, pregnancies in various animal species can show gradations in many features including the site of fertilization, the exact location and duration of embryonic incubation within or near the parent, the size of a brood, the mechanism and magnitude of material exchange between the pregnant adult and embryos, and even the sex of the gestating parent. The wide variety of ways and means by which parents nurture early lifestages of their progeny adds spice to scientific studies of pregnancy and related incubational phenomena.

(5) Internal male-pregnancy affords mirror-image vantages on sexual selection and mating systems

Males (rather than females) become pregnant in all of the more than 200 extant species of pipefishes and seahorses (Syngnathidae). The process begins when a gravid female transfers some or all of her many ova to the male's abdomen or tail, where the eggs either are glued onto his external surface or deposited in a specialized pouch that evolved expressly for this purpose. In species with pouches, the male then fertilizes the clutch internally, seals the pouch, and carries the embryos for several weeks before giving birth to live young. During this pregnancy, the sire nourishes, aerates, osmoregulates and protects his brood whereas the mother provides no care for her offspring. To evaluate the evolutionary history and selective consequences of male-pregnancy in syngnathids, researchers have employed PCM (Avise, 2006), with the results often interpreted in conjunction with findings from extensive molecular parentage analyses of genetic mating systems (Jones & Avise, 2001).

The PCM analyses uncovered a good agreement between clade membership and brood-pouch morphology and generally were consistent with the hypothesis that brood pouches with simple designs evolutionarily predated pouches with more complex architectures (Wilson *et al*., 2003). Results from the genetic parentage analyses of broods confirmed (as expected) that pregnant males invariably have sired the embryos that they carry. Furthermore, these findings coupled with genetic appraisals of maternity helped to confirm the following: (1) many (but not all) syngnathids are 'sex-role-reversed' (Vincent *et al*., 1992; Jones *et al*., 2005) in the sense that sexual selection operates more strongly on females than on males (Jones *et al*., 2000); (2) the direction and intensity of sexual selection generally match expectations based on genetic mating systems that proved to range from monogamy to polygynandry to polyandry in various syngnathid species (Jones & Avise, 2001); and (3) all of these outcomes regarding mating behaviors, sexual dimorphism and sexual selection in the male-pregnant fishes differ diametrically from those that typify female-pregnant fishes and many other vertebrate taxa with more 'conventional' sex roles.

(6) External male-pregnancy offers even more vantages on sexual selection and mating systems

Approximately 89 of 422 taxonomic families of bony fish (21%) contain at least some species with parental care of offspring, and in nearly 70% of such cases the primary or exclusive parental custodian is the male (Blumer, 1979, 1982). Apart from the syngnathid fishes with internal male-pregnancy, parental care in fish species entails phenomena such as nesting, oral brooding and egg-toting, all of which in effect can be thought of as modes of 'external pregnancy' because they too imply a substantial energetic investment in offspring by members of the brooding sex.

Exclusive paternal care of offspring is otherwise quite uncommon in the biological world, so fish again offer mirror-image evolutionary perspectives on parental investment compared with many other animal groups in which females typically are the primary caregivers (Clutton-Brock, 1991). However, an added complication for species with external (as opposed to internal) male-pregnancy is that a bourgeois or nest-tending male sometimes might be cuckolded via 'extra-pair' fertilization events (DeWoody & Avise, 2001). Genetic markers as applied to embryos in the nests of many nest-tending fish species have confirmed that foster parentage is indeed common and can arise via several routes including 'stolen fertilizations' by sneaker or satellite males (DeWoody *et al*., 1998, 2000; Neff, 2001) as well as by egg thievery (Jones, Östlund-Nilsson & Avise, 1998) and/or nest piracy. Genetic parentage analyses in nest-tending fish species similarly have been used to address many additional reproductive phenomena including egg mimicry and female choice of mates (Porter, Fiumera & Avise, 2002), filial cannibalism (DeWoody *et al*., 2001), and alternative reproductive tactics by females as well as by males (Taborsky, 1994; Gross, 1996; Henson & Warner, 1997).

(7) Rates of polygamy are logistically constrained

Evolutionary biologists ever since Bateman (1948) have appreciated that members of the non-pregnant or non-gravid sex (usually males) tend to evolve behavioral dispositions to seek copulations with members of the pregnant or gravid gender (usually females). Thus, when molecular markers were introduced to mating-system analyses in the 1970s, many researchers were intrigued by what they interpreted to be unexpectedly high rates of polygamy in many species suspected from field observations to be mostly monogamous (reviews in Burke, 1989; Avise, 1994; Griffith, Owens & Thuman, 2002). In particular, a research tradition arose wherein a primary goal was to explain why multiple mating by females (polyandry) was far more common that previously thought. For example, as Birkhead (2010) noted in his inaugural THH review, 'The major unanswered question in post-copulatory sexual selection is the adaptive significance of

female promiscuity'. Many hypotheses were advanced and tested in numerous taxa regarding possible direct and indirect fitness benefits that females might derive from polyandry (e.g. Keller & Reeve, 1995; Yasui, 1998; Jennions & Petrie, 2000; Möller & Jennions, 2001). Of course, multiple mating was recognized to have potential downsides as well (such as the risk of contracting sexually transmitted diseases), but overall the bulk of the research effort went into understanding why females (in addition to males) often take multiple mates.

Recent surveys of the literature on genetic parentage in 'pregnant' vertebrate and invertebrate animals (Avise & Liu, 2010, 2011; Avise, Tatarenkov & Liu, 2011) have confirmed that the majority of broods do indeed consist of multiple full-sib cohorts, meaning that a gestating parent typically had several successful mates. Much more surprising, however, were two additional genetic observations: (1) the overall numbers and frequency distributions of mates per brood proved to be remarkably similar across invertebrate and vertebrate taxa; and (2) numbers of mates per pregnancy (typically about 2–5) were much lower than they theoretically could have been given the resolving powers of the molecular markers employed and given the large brood sizes (often with dozens to thousands of embryos) in many of the species assayed. The authors of these review articles concluded that the explanation probably has to do not only with the balance between the costs and benefits of multiple mating but also with the finite logistical opportunities for successful mating events during each breeding season or episode.

Depending on the species, constraints on mate acquisition might include ecological and natural-history factors such as low population densities, short mating seasons, poor vagilities, lengthy courtships, and perhaps even post-copulatory phenomena such as sperm competition and cryptic female choice of sperm (Birkhead & Pizzari, 2002; Eberhard, 2009), the net effect being to truncate mate numbers even in animal species with huge broods and high frequencies of polygamy. Such mating-constraint hypotheses can be viewed as null models for reproductive behaviors in nature (Hubbell & Johnson, 1987; Gowaty & Hubbell, 2009), in which case logistical considerations offer a different perspective on mating systems that might help to counterbalance traditional interpretations based on polyandry's purported selective advantages. For example, before invoking a selective explanation for genetic polygamy in any focal species, an important question might be whether the mean number of successful mates per brooder statistically exceeds or does not exceed the suspected rate of mate encounters given each species' particular biology and ecology.

Synopsis

This essay, in honor of THH, was meant to encapsulate some of the many novel perspectives on evolution offered by creatures that display various forms of clonality (Avise, 2008), hermaphroditism (Avise, 2010) and pregnancy (Avise, 2012). The broadest take-home messages from this collective body of genetic and natural-history evidence are twofold: (1) organismal reproduction is a fascinating topic; and (2) exceptions to biological norms often prove, challenge or otherwise clarify the evolutionary ground rules by which Mother Nature and Father Time generally operate.

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